CLAIMS

1. Nucleotide sequence of sequence SEQ ID No. 1 of the genome of PWD circovirus.

2. Nucleotide sequence of PWD circovirus, characterized in that it is selected from:

a) a nucleotide sequence of a specific fragment of the sequence SEQ ID No. 1;

b) a nucleotide sequence homologous to a nucleotide sequence such as defined in a);

c) a nucleotide sequence complementary to the sequence SEQ ID No. 1 or complementary to a nucleotide sequence such as defined in a) or b), and a nucleotide sequence of their corresponding RNA;

d) a nucleotide sequence capable of hybridizing under stringent conditions with a sequence such as defined in a), b) or c);

e) a nucleotide sequence comprising the sequence SEQ ID No. 1 or a sequence such as defined in a), b), c) or d); and

f) a nucleotide sequence modified by a nucleotide sequence such as defined in a), b), c), d) or e).

3. Nucleotide sequence according to Claim 2, characterized in that it is selected from the sequences ORF1 to ORF3.

4. Nucleotide sequence according to Claim 2, characterized in that it comprises a nucleotide sequence selected from:

a) a nucleotide sequence ORF1, ORF2 or ORF3 according to Claim 3;

b) a nucleotide sequence of a specific fragment of a sequence ORF1, ORF2 or ORF3 according to Claim 3 or a sequence such as defined in a);

c) a homologous nucleotide sequence having at least 80% identity with a nucleotide sequence ORF1, ORF2 or ORF3 according to Claim 3 or such as defined in a) or b);

d) a complementary nucleotide sequence or sequence of RNA corresponding to a sequence ORF1, ORF2 or ORF3 according to Claim 3 or such as defined in a), b) or c); and

e) a nucleotide sequence modified by a sequence ORF1, ORF2 or ORF3 according to Claim 3 or such as defined in a), b), c) or d).

5. Nucleotide sequence according to one of Claims 2 to 4, characterized in that the specific fragment nucleotide sequence comprises a nucleotide sequence selected from the following sequences:

a) 5' TGTGGCGA 3';
b) 5' AGTTTCCT 3';
c) 5' TCATTTAGAGGGTCTTTCAG 3';
d) 5' GTCAACCT 3';
e) 5' GTGGTTGC 3';
f) 5' AGCCCAGG 3';
g) 5' TTGGCTGG 3';
h) 5' TCTAGCTCTGGT 3';
i) 5' ATCTCAGCTCGT 3';
j) 5' TGTCCTCCTCTT 3';
k) 5' TCTCTAGA 3';
l) 5' TGTACCAA 3';
m) 5' TCCGTCTT 3';

and their complementary sequence.

6. Polypeptide encoded by a nucleotide sequence according to one of Claims 1 to 5.

7. Polypeptide according to Claim 6, characterized in that its sequence is represented by a specific fragment of one of the six sequences of amino acids shown in Figure 3.

8. Polypeptide according to Claim 7, characterized in that it is selected from the sequences SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4.

9. Polypeptide characterized in that it comprises a polypeptide selected from:

a) a polypeptide according to Claim 8;

b) a specific fragment of at least 5 amino acids of a polypeptide according to Claim 8, or such as defined in a);

c) a polypeptide homologous to a polypeptide according to Claim 8, or such as defined in a) or b);

d) a specific biologically active fragment of a polypeptide according to Claim 8, or such as defined in a), b) or c); and

e) a polypeptide modified by a polypeptide according to Claim 8, or such as defined in a), b), c) or d).

10. Nucleotide sequence coding for a polypeptide according to Claim 9.

11. Nucleotide sequence utilizable as a primer or probe, characterized in that said sequence is selected from the nucleotide sequences according to one of Claims 1 to 5 and 10.

12. Nucleotide sequence according to Claim 11, characterized in that said sequence is one of the primer of the pairs of primers selected from the following pairs:

a) 5' GTG TGC TCG ACA TTG GTG TG 3', and
5' TGG AAT GTT AAC GAG CTG AG 3';

b) 5' GTG TGC TCG ACA TTG GTG TG 3', and
5' CTC GCA GCC ATC TTG GAA TG 3';

c) 5' CGC GCG TAA TAC GAC TCA CT 3', and
5' GTG TGC TCG ACA TTG GTG TG 3';

d) 5' CGC GCG TAA TAC GAC TCA CT 3', and
5' CTC GCA GCC ATC TTG GAA TG 3'.

13. Nucleotide sequence according to one of Claims 11 to 12, characterized in that it is labeled by a radioactive compound or by a nonradioactive compound.

14. Nucleotide sequence according to one of Claims 11 to 13, characterized in that it is covalently or noncovalently immobilized on a support.

15. Nucleotide sequence according to one of Claims 10 to 12, for the detection and/or the amplification of nucleic sequences.

16. Cloning and/or expression vector, characterized in that it contains a nucleotide sequence according to one of Claims 1 to 5 and 10.

17. Vector characterized in that it comprises a nucleotide sequence according to one of Claims 1 to 5 and 10, and in that it additionally comprises a gene of interest.

18. Viral pseudoparticle or particle generated from a vector according to one of Claims 16 to [sic] 17.

19. Host cell, characterized in that it is transformed by a vector according to one of Claims 16 to [sic] 17, or a viral particle according to Claim 18.

20. Animal, comprising a cell transformed according to Claim 19.

21. Procedure for preparation of a polypeptide, characterized in that it employs a vector according to one of Claims 16 and 17, a cell transformed by said vector and/or an animal comprising said transformed cell.

22. Recombinant polypeptide capable of being obtained by a procedure according to Claim 21.

23. Procedure for preparation of a synthetic polypeptide, characterized in that it uses an amino acid sequence of a polypeptide according to one of Claims 6 to 9.

24. Synthetic polypeptide obtained by a procedure according to Claim 23.

25. Hybrid polypeptide, characterized in that it contains at least the sequence of a polypeptide according to one of Claims 6 to 9, 22 and 24, and a sequence of a polypeptide capable of inducing an immune response in man or animals.

26. Hybrid polypeptide according to Claim 25, characterized in that it contains at least the sequence of a polypeptide according to one of Claims 6 to 9, 22

and 24, and a sequence of a polypeptide capable of inducing a humoral and/or cellular response in man or animals.

27. Nucleotide sequence coding for a hybrid polypeptide according to one of Claims 25 and 26.

28. Vector characterized in that it contains a nucleotide sequence according to Claim 27.

29. Hybrid polypeptide according to one of Claims 25 and 26, characterized in that it is a recombinant polypeptide obtained by the employment of a vector according to Claim 28.

30. Procedure for the detection and/or the identification of PWD circovirus in a biological sample, characterized in that it comprises the following steps:

a) contacting of the biological sample with a polypeptide according to one of Claims 6 to 9, 22 and 24;

b) demonstration of the antigen-antibody complex possibly formed.

31. Kit or set for the detection and/or the identification of PWD circovirus, characterized in that it comprises the following elements:

a) a polypeptide according to one of Claims 6 to 9, 22 and 24;

b) if need be, the reagents for the formation of the medium favorable to the immunological reaction;

c) the reagents allowing demonstration of the antigen-antibody complexes possibly formed between the polypeptide(s) of the invention and the antibodies;

d) if need be, a biological reference sample (negative control) devoid of antibodies recognized by said polypeptide;

e) if need be, a biological reference sample (positive control) containing a predetermined quantity of antibodies recognized by said polypeptide.

32. Mono- or polyclonal antibodies, their fragments, or chimeric antibodies, characterized in that they are capable of specifically recognizing a polypeptide according to one of Claims 6 to 2, 22 and 24.

33. Antibody according to Claim 32, characterized in that it is a labeled antibody.

34. Procedure for the detection and/or the identification of PWD circovirus in a biological sample, characterized in that it comprises the following steps:

a) contacting of the biological sample with an antibody according to one of Claims 32 or 33;

b) demonstration of the antigen-antibody complex formed.

35. Kit or set for the detection and/or the identification of PWD circovirus, characterized in that it comprises the following elements:

a) a polyclonal or monoclonal antibody according to one of Claims 32 or 33;

b) if need be, the reagents for the formation of the medium favorable to the immunological reaction;

c) the reagents allowing the demonstration of the antigen-antibody complexes produced by the immunological reaction.

36. Procedure for detection and/or identification of PWD circovirus in a biological sample, characterized in that it employs a nucleotide sequence according to one of Claims 11 to 15.

37. Procedure according to Claim 36, characterized in that it contains the following steps:

a) if need be, isolation of the DNA from the biological sample to be analyzed;

b) specific amplification of the DNA of PWD circovirus with the aid of at least one primer according to one of Claims 11 to 15;

c) demonstration of the amplification products.

38. Procedure according to Claim 36, characterized in that it comprises the following steps:

a) contacting of a nucleotide probe according to one of Claims 11 to 15 with a biological sample, the DNA contained in the biological sample having, if need be, previously been made accessible to hybridization under conditions allowing the hybridization of the probe with the DNA of the sample;

b) demonstration of the hybrid possibly formed between the nucleotide probe and the DNA of the biological sample.

39. Procedure according to Claim 36, characterized in that it comprises the following steps:

a) contacting of a nucleotide probe immobilized on a support according to Claim 14 with a biological sample, the DNA of the sample having, if need be, previously been made accessible to hybridization under conditions allowing the hybridization of the probe with the DNA of the sample;

b) contacting of the hybrid formed between the nucleotide probe immobilized on a support and the DNA contained in the biological sample, if need be after elimination of the DNA of the biological sample which has not hybridized with the probe, with a nucleotide probe labeled according to Claim 13;

c) demonstration of the novel hybrid formed in step b).

40. Procedure according to Claim 39, characterized in that, previously to step a), the DNA of the biological sample is amplified with the aid of at least one primer according to one of Claims 11 to 15.

41. Kit or set for the detection and/or the identification of associated PWD circovirus, characterized in that it comprises the following elements:

a) a nucleotide probe according to one of Claims 11 to 15;
b) if need be, the reagents necessary for the carrying out of a hybridization reaction;
c) if need be, at least one primer according to one of Claims 11 to 15, as well as the reagents necessary for an amplification reaction of the DNA.

42. Kit or set for the detection and/or the identification of PWD circovirus, characterized in that it comprises the following elements:
a) a nucleotide probe, a so-called capture probe, according to Claim 14;
b) an oligonucleotide probe, called a revealing probe, according to Claim 13;
c) if need be, at least one primer according to one of Claims 11 to 15, as well as the reagents necessary for an amplification reaction of the DNA.

43. Kit or set for the detection and/or the identification of PWD circovirus, characterized in that it comprises the following elements:
a) at least one primer according to one of Claims 11 to 15;
b) if need be, the reagents necessary for carrying out a DNA amplification reaction;
c) if need be, a component allowing the sequence of the amplified fragment to be verified, more particularly an oligonucleotide probe according to one of Claims 11 to 15.

44. Procedure or kit or set according to one of Claims 30 to 43, for the diagnosis of an infection by the PWD circovirus.

45. Use of a nucleotide sequence according to one of Claims 1 to 5 and 10, of a polypeptide according to one of Claims 6 to 9, 22 and 24, of an antibody according to one of Claims 32 and 33, of a cell according to Claim 19, and/or of an animal transformed

according to Claim 20, for the selection of organic or inorganic compounds capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of the PWD circovirus or capable of inducing or of inhibiting in pigs the pathologies linked to an infection by the PWD circovirus.

46. Method for selecting a compound capable of binding to a polypeptide according to one of Claims 6 to 9, 22 and 24, capable of binding to a nucleotide sequence according to one of Claims 1 to 5 and 10, or capable of recognizing an antibody according to Claim 32, and/or capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of the PWD circovirus, or capable of inducing or inhibiting in pigs the pathologies linked to an infection by the PWD circovirus, characterized in that it comprises the following steps:

a) contacting of said compound with said polypeptide, said nucleotide sequence, with a cell transformed according to Claim 19, and/or administration of said compound to an animal transformed according to Claim 20;

b) determination of the activity of said compound.

47. Compound capable of being selected by a method according to Claim 46.

48. Pharmaceutical composition comprising a compound selected from the following compounds:

a) a nucleotide sequence according to one of Claims 1 to 5 and 10;

b) a polypeptide according to one of Claims 6 to 9, 22, 24 to 26 and 29;

c) a vector or a viral particle according to one of Claims 16 to 18 and 28, or a cell according to Claim 19;

d) an antibody according to Claim 32; and

e) a compound according to Claim 47.

49. Composition according to Claim 48, possibly in combination with a pharmaceutically acceptable vehicle.

50. Pharmaceutical composition according to one of Claims 48 and 49, for the prevention or the treatment of an infection by the PWD circovirus.

51. Vaccine composition, characterized in that it comprises one or more polypeptides according to one of Claims 6 to 9, 22 and 24 and/or one or more hybrid polypeptides according to one of Claims 25, 26 and 29.

52. Use of a cell according to Claim 19, for the preparation of a vaccine composition.

53. Vaccine composition, characterized in that it contains a nucleotide sequence according to one of Claims 1 to 5, 10 and 27, a vector according to one of Claims 16, 17 and 28, and/or a cell according to Claim 19.

54. Vaccine composition according to one of Claims 51 and 53 for the prevention or the treatment of an infection by the PWD circovirus.

55. Vaccine composition according to one of Claims 51, 53 and 54, in combination with a pharmaceutically acceptable vehicle and, if need be, one or more appropriate adjuvants of immunity.

56. Vector according to Claim 17, viral particle according to Claim 18, or cell according to Claim 19, for the treatment and/or the prevention of a disease by gene therapy.

57. Pharmaceutical composition comprising, as therapeutic or prophylactic agent, a vector according to Claim 17, a viral particle according to Claim 18 or a cell according to Claim 19.

PATENT

GENOMIC SEQUENCE AND POLYPEPTIDES OF THE CIRCOVIRUS ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD), APPLICATIONS TO THE DIAGNOSIS, PREVENTION AND/OR TREATMENT OF THE INFECTION

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ABSTRACT

The invention relates to the genomic sequence and nucleotide sequences coding for polypeptides of PWD circovirus, such as the structural and nonstructural polypeptides of said circovirus, as well as vectors including said sequences and cells or animals transformed by these vectors. The invention likewise relates to methods for detecting these nucleic acids or polypeptides and kits for diagnosing infection by the PWD circovirus. The invention is also directed at a method for selecting compounds capable of modulating the viral infection. The invention finally comprises pharmaceutical, especially vaccine, compositions for the prevention and/or the treatment of viral infections by PWD circovirus as well as the use of a vector according to the invention for the prevention and/or treatment of diseases by gene therapy.